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(54) **Detector and screening device for ion channels**

(57) The invention provides for a detector assembly,
fiber assembly and screening system for optical meas-
urements.

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Description

[0001] This application claims the benefit of an earlier filing date under 35 U.S.C. §120 to patent application entitled "Detector and Screening Device for Ion Channels" Tsien et al., filed July 17, 1998, docket number 08366/006001, of which the present application is a continuation in part of application, which is herein incorporated by reference.

FIELD OF THE INVENTION

[0002] The present invention generally relates to devices and methods for rapidly identifying chemicals with biological activity in liquid samples, particularly automated screening of low volume samples for new medicines, agrochemicals, or cosmetics.

BACKGROUND

[0003] Drug discovery is a highly time dependent and critical process in which significant improvements in methodology can dramatically improve the pace at which a useful chemical becomes a validated lead, and ultimately forms the basis for the development of a drug. In many cases the eventual value of a useful drug is set by the timing of its arrival into the market place, and the length of time the drug enjoys as an exclusive treatment for a specific ailment.

[0004] A major challenge for major pharmaceutical companies is to improve the speed and efficiency of this process while at the same time maintaining costs to an absolute minimum. One solution to this problem has been to develop high throughput screening systems that enable the rapid analysis of many thousands of chemical compounds per 24 hours. To reduce the otherwise prohibitive costs of screening such large numbers of compounds, typically these systems use miniaturized assay systems that dramatically reduce reagent costs, and improve productivity. To efficiently handle large numbers of miniaturized assays it is necessary to implement automatic robotically controlled analysis systems that can provide reliable reagent addition and manipulations. Preferably these systems and the invention herein are capable of interacting in a coordinated fashion with other systems sub-components, such as a central compound store to enable rapid and efficient processing of samples.

[0005] Miniaturized high throughput screening systems require robust, reliable and reproducible methods of analysis that are sensitive enough to work with small sample sizes. While there are a large number of potential analysis methods that can successfully used in macroscopic analysis, many of these procedures are not easily miniaturizable, or lack sufficient sensitivity when miniaturized. This is typically true because absolute signal intensity from a given sample decreases as a function of the size of the sample, whereas background optical or detector noise remains more or less constant for large or small samples. Preferred assays for miniaturized high throughput screening assays have a high signal to noise ratios at very low sample sizes.

[0006] Fluorescence based measurements have high sensitivity and perform well with small samples, where factors such as inner filtering of excitation and emission light are reduced. Fluorescence therefore exhibit good signal to noise ratios even with small sample sizes. A particularly preferred method of using fluorescence based signal detection is to generate a fluorescent (emission) signal that simultaneously changes at two or more wavelengths. A ratio can be calculated based on the emission light intensity at the first wavelength divided by the emitted light intensity at a second wavelength. This use of this ratio to measure a fluorescent assay has several important advantages over other non-ratiometric types of analysis. Firstly, the ratio is largely independent on the actual concentration of the fluorescent dye that is emitting fluorescence. Secondly, the ratio is largely independent on the intensity of light with which the fluorescent compound is being excited. Thirdly, the ratio is largely independent of changes in the sensitivity of the detector, provided that is that these changes are the same for the detection efficiency at both wavelengths. This combination of advantages make fluorescence based ratiometric assays highly attractive for high throughput screening systems, where day to day, and, assay to assay reproducibility are important.

[0007] Fluorescence assays that produce ratiometric emission readouts have gained in popularity as the advantages of the method have grown in acceptance. Changes in emission ratios at two more wavelengths can be created through a number of distinct mechanisms including electronic and conformational changes in a fluorescence compound. Typically, these changes can occur in response to a chemical reaction or binding of the fluorescent compound to a particular ion such as a metal ion like calcium or magnesium, or through a change in pH that influences the protonation state of the fluorescent compound.

[0008] Alternatively ratiometric changes in emission can be conveniently be obtained by exploiting the use of fluorescence resonance energy transfer (FRET) from one fluorescent species to another fluorescent species. This approach is predictable, sensitive and can give rise to large ratio changes at two well-defined and well spectrally resolved wavelengths. Furthermore FRET can be generally applied to create ratiometric assays for a range of activities. For example patent WO 96/30540 (Tsien) describes a FRET based system to measure gene expression using a fluorogenic substrate of beta lactamase. Patent WO 96/41166 (Tsien) describes the use of a FRET based system to measure voltage

across the plasma membrane of a cell. Patent WO 97/20261 (Tsien) describes the use of FRET between two fluorescent proteins to measure intracellular protein. Such assays can be used with the inventions described herein.

[0009] The present invention is directed towards the development of improved optical systems and methods for simultaneously measuring emission ratios from a plurality of samples with high sensitivity, speed, reproducibility and accuracy. The present invention has several important advantages over prior methods and devices adapted to measure fluorescence emission sequentially from samples.

Firstly, the simultaneous measurement of emission ratios enables rapid fluctuations in lamp intensity, bleaching of the fluorescent dye, or cycle to cycle errors in the alignment of multiwell plates to be corrected for, thereby enabling much smaller changes in ratio to be reliably observed. Secondly, no mechanical movements are necessary during ratio measurement, eliminating mechanical design challenges. Thirdly ratios can be acquired very rapidly, as required for dynamic measurements of membrane potential or calcium, and are not limited by the speed of filter changing. Fourthly the overall throughput and duty cycle are improved by eliminating dead times for filter changeover. Finally, residual ratio non-uniformities between addressable wells should be constant and easily correctable by using emission ratios previously measured on reference samples to normalize sample ratios in software.

SUMMARY OF THE INVENTION

[0010] The invention includes a method of simultaneously measuring at least two optical properties of emitted light from at least one sample in a plurality of addressable wells of a multiwell plate, comprising the steps of,

- i) aligning a plurality of addressable wells of a multiwell plate to a plurality of ball lenses;
- ii) directing electromagnetic radiation substantially coaxially through the symmetry axis of each of said plurality of ball lenses,
- iii) detecting the emitted light focused by said plurality of ball lenses from said at least one sample.

[0011] The invention includes an optical detection system, comprising a light source that launches at least one predetermined wavelength of light, sample holder, a ball lens at a predetermined interrogation distance from said sample holder, a trifurcated fiber adapted for dual optical interrogation and in optical communication with said ball lens, and a detector that detects light of at least one desired wavelength and in optical communication with said ball lens. Typically, the optical detection system includes a trifurcated fiber comprising a first plurality of emission bundles for receiving light of a first wavelength and second plurality of emission bundles for receiving light of a second wavelength and said first plurality of emission bundles and said light source launches at least one predetermined wavelength of excitation light at said sample holder. The optical detection system may further comprise at least one positioner to controllably change the spatial relationship between the ball lens and the fiber or sample or multiwell plate or a combination thereof. Typically, the light source launches light through said trifurcated fiber to the location at least one addressable well in a sample in said sample holder to monitor epifluorescence. Preferably, the trifurcated fiber comprises an end that is generally at a focal plane of the ball lens.

[0012] The invention also includes an optical fiber assembly, comprising a trifurcated fiber comprising a first plurality of emission bundles for receiving light of a first wavelength and second plurality of emission bundles for receiving light of a second wavelength and said first plurality of emission bundles and said second plurality of emission bundles are non-randomly distributed in plurality of excitation bundles.

BRIEF DESCRIPTION OF THE FIGURES

[0013]

FIG. 1 shows one embodiment of a fluorescence measuring device utilizing the detection system of the invention.

FIG. 2 shows one embodiment of a detection arrangement according to the invention.

FIG. 3 shows a cross sectional view of the trifurcated fiber optic bundles showing potential arrangements of the individual fiber optic fibers. Excitation fibers being represented by X or cross hatching, and emission fibers being represented by the letters (A) for the first emission leg of a trifurcated fiber optic bundle, and (B), for the second emission leg of a trifurcated fiber optic bundle.

FIG. 4 shows several embodiments of a ball lens of the invention in a cross sectional view depicting the light directing ability of the lens to focus light from the sample, 400 and 401 to the fiber optic bundle face plate 402. FIG 4A, depicts a 5 mm sapphire ball lens spaced 1 mm from the sample, FIG 4B, depicts a 10 mm glass ball lens spaced 1 mm from the sample and FIG 4C, depicts a 20 mm glass ball lens spaced 1 mm from the sample.

FIG. 5 shows a perspective view of one embodiment of the ball lens assemblies of the present invention. The ball lens 500, is engaged by a ball lens holding assembly, 501 & 502, and spring 503 to maintain accurate alignment of

the ball lens and fiber optic bundle 504. The mounting assembly for the assembly 505 mounts the assembly to the z-axis mover (see FIG.6)

FIG. 6 shows a perspective view of one embodiment of a ball lens assembly Z-axis mover according to the invention. The stepper motor 600, z-axis mounting assembly 601, cam 602 and 603, ball lens assemblies 604, platform for ball lens assemblies 605, guiding pillar 606, switch 607, and trifurcated fiber optic bundle 608.

FIG. 7 shows a perspective view of one embodiment of a filter changer of the invention. The filter holder enclosure 700 & 701, filter holder support 702 & 703, trifurcated fiber optic assembly 704, photomultiplier (PMT) 705, support 706, holding platform 707, and light tight O-ring 708.

FIG.8A shows the rapid detection and continuous analysis of voltage changes induced within a cell measured using one preferred embodiment of the invention.

FIG.8B shows a dose response curve of voltage changes induced within a cell measured in response to the addition of an ion channel blocker, using one preferred embodiment of the invention.

FIG. 9 shows the use of one embodiment of a device comprising the trifurcated ball lens assemblies of the invention to screen for ligand gated ion channel receptor antagonists.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

[0014] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention belongs. Generally, the nomenclature used herein and many of the automation, computer, detection, chemistry and laboratory procedures described below are those well known and commonly employed in the art. Standard techniques are usually used for engineering, robotics, optics, molecular biology, computer software and integration. Generally, chemical reactions, cell assays and enzymatic reactions are performed according to the manufacture's specifications where appropriate. The techniques and procedures are generally performed according to conventional methods in the art and various general references (see generally Lakowicz, J.R. *Principles of fluorescence spectroscopy*, New York:Plenum press (1983), and Lakowicz, J. R. *Emerging applications of fluorescence spectroscopy to cellular imaging: lifetime imaging metal-ligand probes, multi-photon excitation and light quenching*. Scanning Microsc Suppl VOL. 10 (1996) pages. 213-24, for fluorescent techniques, Sambrook et al *Molecular Cloning: A laboratory manual*, 2nd ed. (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. for molecular biology methods, Optics Guide 5 Melles Griot® Irvine CA for general optical methods, Optical Waveguide Theory, Snyder & Love published by Chapman & Hall, and Fiber Optics Devices and Systems by Peter Cheo, published by Prentice-Hall for fiber optic theory and materials.

[0015] As employed throughout the disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

[0016] "Multiwell plate" refers to a two dimensional array of addressable wells located on a substantially flat surface. Multiwell plates may comprise any number of discrete addressable wells, and comprise addressable wells of any width or depth. Common examples of multiwell plates include 96 well plates, 384 well plates and 3456 well nanoplates.

[0017] "Addressable well" refers to spatially distinct location on a multiwell plate that may or may not have a physical representation outside of the computer representation of the plate.

[0018] "Chemical plate" refers to a multiwell plate containing chemicals, such as stock solutions or dilutions thereof.

[0019] "Pharmaceutical agent or drug" refers to a chemical compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient.

[0020] As used herein, "optical property" refers a measurable property of light, such as the intensity of emission light at a particular wavelength, the intensity or degree of light polarization, the transmittance of a compound or composition, or the reflectance of a compound or composition.

[0021] "Ball lens" refers to a sphere, truncated sphere, cylinder, or truncated cylinder of suitable transparent refractive material and is usually a sphere.

[0022] "Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner.

[0023] "Optical Interrogation" means the process of detecting, or measuring at least one optical property of a sample by at least one detection device. A detection device typically would comprise a photon detection device such as a photon multiplier tube (PMT).

DESCRIPTION OF ONE EMBODIMENT OF THE INVENTION

[0024] FIG. 1 shows one device of the invention. In one embodiment of the invention, a device integrates a liquid handler 115, a multiwell positioning stage 112 and a detection device comprising the ball lens trifurcated fiber optic bundle

ball lens assembly of the invention. The vertical position of the optical assembly can be adjusted by a stepper motor driven cam system (further described in FIG. 6). The assemblies are lowered when the plate is moved in or out of the system to allow the skirt of the microplate to pass over the trifurcated fiber optic bundle ball lens assembly. The assemblies (further described in FIG. 5) are raised once the plate is in the system to maximize fluorescence detection efficiency. Plates containing cells and compounds are loaded into the device either manually or by a computer-controlled arm. The device then takes the plate(s) into the light-tight reading area 116. A multiwell plate positioning stage 112, such as a 500000 series, Parker Hannifin Corp, Harrison City, PA, may also be used to control movement of the multiwell plate. In one embodiment, the liquid handler 115 may be a modified Hamilton Micro Lab 2000 MPH, Hamilton Co, Reno, NV, with at least 1 dispensing tip 114 and associated pump 107, waste container 108 and diluent container 109. The detector module 111, comprises 16 photomultipliers, (Hamamatsu HC124-01) that are used to detect fluorescence emission at a rate of 1Hz or 10Hz. Two photomultiplier tubes are used to detect fluorescence from each well in a column of 8 wells allowing for continuous emission ratio detection. The blue-sensitive bi-alkali photomultiplier tube is typically used to detect the shorter wavelength emission (300 to 650 nm) while the multi-alkali photomultiplier tube is used to detect longer wavelength emission (300 to 850 nm). A computer 105, and graphical user interface 101 coordinates the functions of the liquid handler 115, multiwell plate positioning stage 112, detector module 111 and data collection 106. Data collection can be viewed through a monitor in real-time via a computer monitor 103. A central power switch can be used to switch the device on and off 104.

[0025] Referring to FIG. 2, monolayers of cells can be detected on the bottom of microplate wells 206 by the common end of a trifurcated optical fiber bundle 203. One leg of the each trifurcated fiber bundle is used as an excitation source 201; each of the eight excitation legs is fused into a single bundle 204 to provide uniform light intensity to each of the eight trifurcated bundles. The other two legs of the trifurcated fiber are used for to detect fluorescence emission 214 and 213. The common end of the trifurcated bundle is used to both excite and collect fluorescence emission. Eight trifurcated fibers are used to detect two emission channels from each well in a column of eight wells. A ball lens 205, (RB-707004, Bird Precision Waltham, MA) may be included at the top of the common end of the trifurcated fiber bundle to increase the efficiency of fluorescence detection.

[0026] A 300 watt xenon arc lamp 201, e.g. CXP300, ILC Technology, Sunnyvale, CA) with a parabolic reflector can be used as the fluorescence excitation source. The excitation light is filtered by two 2" diameter interference filters (e.g. 400RDF15 or 480RDF20, Omega Optical, Brattleboro, VT) and then focused by a lens 202 on to the excitation leg of the trifurcated bundle. Both a IR heat absorbing water filter 208 and shutter system 207 are also included in the optical path to protect the interference filters from heat damage. A 1" diameter "head-on" photomultiplier (e.g. HC124 series, Hamamatsu Corp. Bridgewater, NJ) tubes are used to detect the fluorescence emission. Fluorescence emission from one leg of the fiber bundle is detected by a blue-sensitive bi-alkali photomultiplier tube 209; emission from the other leg of the fiber bundle is detected by a red-sensitive multi-alkali photomultiplier tube 210. Data is collected by the A/D portion of a multifunction board (e.g. PCI-MIO-E-1, National Instruments, Dallas, TX) in a Pentium™ based personal computer 212. The computer controls data acquisition, plate and fiber movement, and shutter opening and closing 215.

COMPONENTS OF THE DETECTION SYSTEM

[0027] Typically, the greatest issue in fluorescent detection is the reduction of background signal in the detection system. In this case the detection system might comprise the excitation source and associated optics (dichroic filters, interference filters, focussing lenses, collimators, etc), the fiber optic assembly (excitation and emission pathways and patterns), the substrate containing sample to be analyzed, and the emission filters and associated optics that direct the emission radiation to the detection element. A key challenge in epifluorescent detection (where the excitation light and emission light are directed and collected from the same plane) is to maximize the excitation light energy and the area (the field of view created by the excitation light) this energy is delivered to the sample, without comprising the efficient collection of the fluorescent emission or generating a high background from the reflection of excitation radiation. Typically, a tradeoff exists between optimal radiant energy, the field of view illuminated by the excitation energy and the fluorescent emission collection efficiency. For example, the wavelength to be utilized for excitation may preclude the use of certain materials (which might have other desirable features like high numerical aperture (NA)) due to the incompatibility of the material (high autofluorescence) with the excitation wavelength that is required. The ultimate sensitivity of fluorescent detectors is thus often limited by the amount, and drift in background noise sources that are mainly generated at the various optical interfaces, where reflection and refraction takes place.

[0028] Typically, a detection system of the invention includes a ball lens in optical communication with a tetra, tri- or bi-furcated fiber optic bundle that is in optical communication with a photon detector. A liquid handling system is included to provide predetermined dispensing at a designated time and volume. Preferably dispensation and optical interrogation are coordinated by computer control. Preferably, a first positioner controls the interrogation distances between a ball lens and sample or sample holder. A second positioner may be included (either with or without the first positioner to control the transmission distance between a ball lens and the fiber bundle.

[0029] *Ball lenses and trifurcated fiber optic assemblies*

[0030] Ball lenses provide a compact, wide field of view lens, that when coupled with a suitable fiber optic bundle arrangement significantly reduces background noise. The ball lens trifurcated fiber optic assemblies of the invention are effective in directing light from the light source into the sample in the addressable well, and of efficiently focusing emitted light from the sample to the emission legs of the trifurcated fiber optic bundle. The light focusing ability of ball lens of various sizes and compositions are shown in FIG 4. Such ball lens, comprising of glass, sapphire or fused silica can collect emission light from up to about 65° from the optical axis, and maintain a high numerical aperture (NA) even with an air gap of 50-100 μm between the apex of the ball lens and bottom of the multiwell plate. Furthermore, vignetting, the variation in lens image intensity between the center to the edge of the image, is minimal across the fiber optic face plate. These advantages are further enhanced when a trifurcated fiber optic bundle is used in conjunction with a ball lens. In one embodiment, a plurality of excitation fibers are coaxially arranged within the trifurcated fiber optic bundle and direct the light substantially through the axis of symmetry of the ball lens. Under these conditions, the excitation light is confined to <11° of the optical axis, therefore the side walls of the addressable wells of the multiwell plate are not illuminated, reducing background scattering and fluorescence. Additionally all reflected light that returns from the multiwell plate with an angle of <11° of the optical axis enters the excitation fibers and not the emission fibers. This sacrifices a small amount of fluorescence but rejects light specularly reflected from both surfaces of the multiwell plate bottom.

[0031] To select the preferred optical components for a specific application it is often preferred to determine the signal to noise or signal to background level for particular combinations of ball lens and fiber optic assemblies. Signal to noise ratios can be determined by comparing the magnitude of a defined amount of fluorescent material measured in the optical system, compared to the noise obtained by measuring an empty well under exactly the same conditions. S/N ratios can be calculated at a range of concentrations of the calibration material (for example, fluorescein) to determine overall detector sensitivity and linearity. Additionally, variability of measurements can be expressed in terms of standard deviation (S.D.) and Coefficient of Variance (C.V.) to establish reproducibility and alignment sensitivity of each of the systems.

[0032] Additionally, it is preferred to select the spacing of the fiber optic bundle to the ball lens and the ball lens to the surface of the object to be optically interrogated. This can be quickly accomplished by generating a graph of S/N ratio versus interrogation or transmission distance for each of the optical arrangements desired. In the same way, similar S/N ratio graphs can be created for each of the combinations in response to different illumination intensities and wavelengths of excitation light (in conjunction with appropriate fluorescent samples). This analysis would create a matrix of performance characteristics as represented by S/N ratios that are used to select the optimal fiber optic assemblies, ball lens size, composition, antireflective coating, and spatial alignments of the components for specific applications.

[0033] For example, fiber optic bundles may be created with varying packing patterns of excitation and emission bundle numbers and arrangements, and with different numbers of fibers in the excitation legs and emission legs. In one embodiment, the packing of the fibers of both the excitation and emission legs in the bundle is randomly packed. In another embodiment the fibers are arranged in specific and defined patterns, that confers a preferred optical characteristic to the system. For example, in one preferred embodiment discussed above, the excitation fibers could be bundled together centrally in the fiber optic bundle and the emission filters arranged around the outside to create a coaxial fiber optic bundle. Both bifurcated and trifurcated fiber bundles can be produced in this preferred configuration. Alternatively, the emission bundles could be arranged in small groups to create an array, or radially around the axis of the bundle, or any other symmetrical or non-symmetrical pattern.

[0034] Fiber optic assemblies may also vary in total number of fibers of both the excitation and emission legs and overall size. The number of excitation fibers and the number of emission fibers and the relative ratios of excitation fibers to emission fibers may be widely varied depending upon the other components in the system, as well as the type of light source, sensitivity of the detector and size of the addressable well in which the sample is located. The optimization of these factors is discussed herein. In one embodiment a fiber optic bundle may contain a total of 341 fibers of which 55 will be excitation fibers arranged randomly within the fiber. In another embodiment the fiber may have 341 fibers of which 85 fibers are excitation fibers arranged in preferentially within the center of the bundle, but also distributed randomly through the remainder of the emission bundles. In another embodiment the fiber may contain 112 fibers of which 7 fibers are excitation fibers arranged in the center of the bundle, and the remaining emission fibers are located around the excitation fibers. In another embodiment the fiber may contain 1417 fibers of which 163 fibers are excitation fibers arranged in the center of the bundle, and the remaining emission fibers are located around the excitation fibers. In another embodiment of the fiber optic bundle, the excitation fibers are centrally located within the bundle and extend beyond the point where the emission fibers terminate. In a preferred version of this embodiment the emission filters terminate into a liquid light guide that is in contact with the ball lens. Typically, the percent of excitation to emission fibers in the trifurcated fiber optic bundle ranges from about 5 to 10 percent excitation fibers, or about 10-20 percent excitation fibers or about 20-40 percent excitation fibers.

[0035] Additional optimization of the composition and size of the ball lens is desirable for each fiber optic bundle

arrangement and application. Ball lens compositions of materials of different refractive index and of different sizes can be easily evaluated with each fiber optic arrangement to establish a preferred optical arrangement. Ball lens of about 1 mm, 2 mm, 3 mm, 4 mm, 5 mm, 6 mm, 8 mm, 10 mm and 20 mm diameter may be evaluated depending on the size of the instrument and spatial requirements of the imaging system desired. Suitable compositions of the ball lens include fused silica, sapphire, optical glass (such as BK7, SF11 or LaSF9), borosilicate glass or zinc selenide (for infrared applications). Preferred compositions of the ball lens for use within the wavelength range 300-750 nm include fused silica and sapphire. For low light applications it is often necessary to include a suitable anti-reflective coating such as single or multi-layer MgF_2 , V-coatings, HEBBAR™ (High Efficiency BroadBand AntiReflection) and Extended range AntiReflective coatings for a ball lens. To determine the optimum composition, size and AntiReflective coating (AR) of the lens different coatings, each size of ball lens above made of each of the materials above would be prepared with each of the AR coatings above, and in the absence of an AR coating and evaluated as described herein.

Detectors

[0036] The detector can include at least one photon sensitive surface or material for measuring photon emission, such as a charged coupled device (CCD), photodiode, or a photomultiplier tube (PMT). The detector can intensify the signal, and gate if desired, using a photon intensifier. Preferably, the detector can utilize a high quantum efficiency CCD without an intensifier for long detection integration. Alternatively, the detector can utilize a plurality of PMT's or multi-site PMT's for simultaneous photon detection and quantitation at two wavelengths from a plurality of addressable wells.

[0037] The detector preferably functions in the epi-fluorescence mode where the preferred illumination is from the bottom of the multiwell plate and the preferred collection is also from the bottom of the multiwell plate. The detector usually is capable of three to four orders of magnitude of dynamic range in signal response from a single reading. The detector, in one embodiment, utilizes a CCD chip for imaging and detecting photons emitted from the assay wells.

Light source

[0038] In the preferred embodiment, the detector comprises a light source assembly (e.g., Xenon lamp) that can be switched (either manually or through computer control) between continuous and pulsed (1kHz) output depending upon power supply. Suitable light sources, for example lasers, light emitting diodes (LEDs) or mercury arc lamps are also suitable as are other light sources that are described herein and other suitable sources can be developed in the future.

Liquid handlers

[0039] In one embodiment, the liquid handler can comprise a plurality of nanoliter pipetting tips that can individually dispense a predetermined volume. Typically, pipetting tips are arranged in two-dimension array to handle plates of different well densities (e.g., 96, 384, 864 and 3,456).

[0040] Usually, the dispensed volume will be less than approximately 2,000 microliters of liquid that has been aspirated from a predetermined selection of addressable wells and dispensed into a predetermined selection of addressable wells. Preferably, nanoliter pipetting tips can dispense less than approximately 500 nanoliters, more preferably less than approximately 100 nanoliters, and most preferably less than approximately 25 nanoliters. Dispensing below 25 nanoliters can be accomplished by pipetting tips described herein. Preferred, minimal volumes dispensed are 5 nanoliters, 500 picoliters, 100 picoliters, 10 picoliters. It is understood that pipetting tips capable of dispensing such minimal volumes are also capable of dispensing greater volumes. The maximal volume dispensed will be largely dependent on the dispense time, reservoir size, tip diameter and pipetting tip type. Maximum volumes dispensed are about 10.0 microliters, 1.0 microliters, and 200 nanoliters. Preferably, such liquid handlers will be capable of both dispensing and aspirating. Usually, a nanoliter pipetting tip (or smaller volume dispenser) comprises a fluid channel to aspirate liquid from a predetermined selection of addressable wells (e.g., chemical wells containing drug candidates). Liquid handlers are further described herein, and for some volumes, typically in the microliter range, suitable liquid pipetting tips known in the art or developed in the future can be used. It will be particularly useful to use liquid handlers capable of handling about 1 to 20 microliter volumes when it is desired to make daughter plates from master plates. Preferably, in such instances a liquid handler has a dispensing nozzle that is adapted for dispensing small volumes and can secure a tip having a fluid reservoir.

[0041] In one embodiment nanoliter pipetting tips comprise solenoid valves fluidly connected to a reservoir for liquid from an addressable chemical well. The fluid reservoir can be a region of a dispenser that can hold fluid aspirated by the nanoliter pipetting tip. Usually, a tip reservoir will hold at least about 100 times the minimal dispensation volume to about 10,000 times the dispensation volume and more preferably about 250,000 times the dispensation volume. The solenoid valves control a positive hydraulic pressure in the reservoir and allow the release of liquid when actuated. A positive pressure for dispensation can be generated by a hydraulic or pneumatic means, e.g., a piston driven by a motor

or gas bottle. A negative pressure for aspiration can be created by a vacuum means (e.g., withdrawal of a piston by a motor). For greater dispensing control, two solenoid valves or more can be used where the valves are in series and fluid communication.

[0042] In another embodiment, nanoliter pipetting tips comprise an electrically sensitive volume displacement unit in fluid communication to a fluid reservoir. Typically, the fluid reservoir holds liquid aspirated from an addressable chemical well. Electrically sensitive volume displacement units are comprised of materials that respond to an electrical current by changing volume. Typically, such materials can be piezo materials suitably configured to respond to an electric current. The electrically sensitive volume displacement unit is in vibrational communication with a dispensing nozzle so that vibration ejects a predetermined volume from the nozzle. Preferably, piezo materials are used in dispensers for volumes less than about 10 to 1 nanoliter, and are capable of dispensing minimal volumes of 500 to 1 picoliter. Piezo pipetting tips can be obtained from Packard Instrument Company, Connecticut, USA (e.g., an accessory for the MultiProbe 104). Such small dispensation volumes permit greater dilution, conserve and reduce liquid handling times.

[0043] In some embodiments, the liquid handler can accommodate bulk dispensation (e.g., for washing). By connecting a bulk dispensation means to the liquid handler, a large volume of a particular solution to be dispensed many times. Such bulk dispensation means, for example a modified Hamilton Micro Lab 2200, (MPH, Hamilton Co, Reno, NV) are known in the art and can be developed in the future.

Positioners, transitional stages

[0044] Interrogation, aspiration or dispensation into multiwell plates of different densities can be accomplished by automated positioning (e.g. orthogonal) of a multiwell plate. Typically, the multiwell plates are securely disposed on an orthogonal positioner that moves the wells of a multiwell plate with a first density in an X,Y position with respect to the X,Y position of the liquid handler. Usually, the liquid handler will have an array of aspiration and/or dispensation heads, or both. Many aspiration/dispensation heads can operate simultaneously. The orthogonal positioner will align each addressable well with the appropriate dispensing head. Preferably, a predetermined location (e.g., center) of a pre-selected addressable well will be aligned with the center of a dispensing head's fluid trajectory. Other alignments can be used, such as those described in the examples. With a head substantially smaller than a well diameter, orthogonal positioning permits aspiration or dispensation into plates of different densities and well diameters.

[0045] An orthogonal positioner can typically match an array of dispensing heads with an array of addressable wells in X,Y using a mechanical means to move the addressable wells into position or the liquid handler (e.g., dispensing heads) into position. Preferably, arrays of addressable wells on a plate are moved rather than the liquid handler. This design often improves reliability, since multiwell plates are usually not as heavy or cumbersome as liquid handlers, which results in less mechanical stress on the orthogonal positioner and greater movement precision. It also promotes faster liquid processing times because the relatively lighter and smaller multiwell plates can be moved more quickly and precisely than a large component. The mechanical means can be a first computer-controlled servo motor that drives a base disposed on a X track and a second computer-controlled servo motor that drives a Y track disposed on the X track. The base can securely dispose a multiwell plate and either a feedback mechanism or an accurate Cartesian mapping system, or both that can be used to properly align addressable wells with heads. Other such devices, as described herein, known in the art or developed in the future to accomplish such tasks can be used. Usually, such devices will have an X,Y location accuracy and precision of at least ± 0.3 mm in X and ± 0.3 mm in Y, preferably of at least ± 0.09 mm in X and ± 0.09 mm in Y, and more preferably of at least ± 0.01 mm in X and ± 0.01 mm in Y. It is desirable that such devices comprise detectors to identify the addressable wells or multiwell plates being orthogonally positioned. Such positioners for predetermined X, Y coordinates can be made using lead screws having an accurate and fine pitch with stepper motors (e.g., Compumotor Stages from Parker, Rohnert Park, CA, USA). Positioners (e.g. X, Y or Z) can be used to move the detector assembly, the sample, liquid handler or a combination thereof.

[0046] Alternatively, the liquid handler can be disposed on a Z-positioner, having an X,Y positioner for the liquid handler in order to enable precise X,Y and Z positioning of the liquid handler (e.g., Linear Drives of United Kingdom).

[0047] A reference point or points (e.g., fiducials) can be included in the set up to ensure that a desired addressable well is properly matched with a desired addressable head. For instance, the multiwell plate, the orthogonal positioner or the liquid handler can include a reference point(s) to guide the X,Y alignment of a plate, and its addressable wells, with respect to the liquid handler. For example, the liquid handler has a detector that corresponds in X,Y to each corner of a plate. The plate has orifices (or marks) that correspond in X,Y to the liquid handler's position detectors. The plate's orifices allow light to pass or reflect from a computer-controlled identification light source located on the orthogonal positioner in the corresponding X,Y position. Optical locators known in the art can also be used in some embodiments (PCT patent application WO91/17445 (Kureshy)). Detection of light by the liquid handler emitted by the orthogonal positioner verifies the alignment of the plates. Once plate alignment is verified, aspiration or dispensation can be triggered to begin. Stepper motors can be controlled for some applications as described in U.S. Patent 5,206,568 (Bjornson).

[0048] The liquid handler will also typically be disposed on a Z-dimensional positioner to permit adjustments in liquid

transfer height. This feature allows for a large range of plate heights and aspirate and dispense tips, if desired, to be used in the sample distribution module. It also permits the dispense distance between a addressable well surface, or liquid surface in an addressable well, and a liquid handler to be adjusted to minimize the affects of static electricity, gravity, air currents and to improve the X,Y precision of dispensation in applications where dispensation of a liquid to a particular location in a addressable well is desired. Alternatively, multiwell plates can be positioned on a Z-dimensional positioner to permit adjustments in liquid transfer height. Static neutralizing devices can also be used to minimize static electricity. Generally, the liquid transfer height will be less than about 2 cm. Preferably, small volumes will be dispensed at a liquid transfer height of less than about 10 mm, and more preferably less than about 2 mm. Occasionally, it may be desirable to contact the tips with a solution in a controllable fashion, as described herein or known in the art

Control of Z-axis movement of ball lens assembly

[0049] The ball lens assembly will also typically be disposed on a Z-dimensional positioner to permit adjustments in interrogation distance and transmission distance. For example through the use of a stepper motor driven cam system or other positioners as described herein. The assemblies are lowered when the plate is moved in or out of the system to allow the skirt of the microplate to pass over the trifurcated fiber optic bundle ball lens assembly. The assemblies may be raised once the plate is in the system to control the interrogation distance to improve fluorescence detection efficiency. Alternatively, multiwell plates can be positioned on a Z-dimensional positioner to permit adjustments in interrogation distance. Typically the transmission distance between the ball lens and fiber optic bundle would be fixed at a preferred distance, for optimal fluorescence detection. In a preferred embodiment, adjustments in transmission distance could be under programmable control to optimize the sensitivity and reproducibility of fluorescence measurements.

Control Data Processing and/or Integration Modules

[0050] In one embodiment, a data processing and integration module can integrate and programmably control a liquid handler module, and a detector module to facilitate rapid processing of the multiwell wells. In a preferred embodiment the data processing and integration module can also control the distance of the ball lens assembly to the sample (the interrogation distance), and the distance of the ball lens to the trifurcated fiber optic bundle (the transmission distance). To manage information in the system, the data processing and integration module comprises elements to store, manage and retrieve data, including a data storage device and a processor. The data storage device can hold a relational database, an array of physical disk drives (e.g., random access disk drives), and a connection to other system components via a network. A data storage device can, for instance, store a relational database for environmental, diagnostic, and drug discovery applications. For instance, one particularly useful relational database can be provided by Oracle, and the network can be a TCP/IP (transfer communication protocol) ethernet LAN (local area network).

Interface designs

[0051] In most embodiments, it will be advantageous to integrate and operably link device of the invention with at least one other workstation, usually a sample transporter. The integration can be accomplished with a computer and associated control programs to instruct the translational stage and sample processor to operate coordinately. Alternatively, the device may be used without directly integrating to another workstation by tracking addressable wells in groups and either mechanically or manually transporting multiwell plates to another workstation where the multiwell plates are identified. For instance, the device of the invention may be directly integrated and operably linked to a storage and retrieval module and sample transporter, and indirectly linked to an integration and control module. While this approach is feasible, especially for lower throughputs, it is not desirable for higher throughputs as it lacks direct integration that can lead to faster throughput times. Manual operations also are more frequently subject to error especially when processing large numbers of samples. Preferably, the device of the invention can be integrated with other workstations and operate in a mode with minimal or substantially no manual intervention related to transferring multiwell plates to other work stations.

Usage modes

[0052] The detector module and its system are often capable of many different operating modes that facilitate drug discovery assay requirements. These operating modes can include: single excitation wavelength with single emission wavelength detection, single excitation wavelength, dual emission wavelength detection, sequential or dual excitation wavelength with dual emission wavelength detection and ratio measurement determination, sequential dual excitation wavelength with four emission wavelength detection and ratio measurement determination, homogeneous time resolved fluorescence with single excitation wavelength and single emission wavelength detection, homogeneous time

resolved fluorescence with single excitation wavelength and dual emission wavelength detection and ratio determination measurement, homogeneous time resolved fluorescence with sequential dual excitation wavelength and dual emission wavelength detection and ratio determination measurement, absorbance (e.g. dual), transmittance (e.g. dual), reflectance, dual sequential excitation wavelengths and single emission wavelength detection with ratio determination measurement, luminescence measurement at a single wavelength with luminescence measurement at dual wavelengths, luminescence measurement at dual wavelengths with a ratio determination, and time resolved fluorescence emission (intrinsic dye properties with or without a binding event).

Fluorescence Measurements

[0053] It is recognized that different types of fluorescent monitoring systems can be used to practice the invention with fluorescent probes, such as fluorescent dyes or substrates. Preferably, systems dedicated to high throughput screening, e.g., 96-well or greater microtiter plates, are used. Methods of performing assays on fluorescent materials are well known in the art and are described in, e.g., Lakowicz, J.R., *Principles of Fluorescence Spectroscopy*, New York: Plenum Press (1983); Herman, B., *Resonance Energy Transfer Microscopy*, in: *Fluorescence Microscopy of Living Cells in Culture, Part B, Methods in Cell Biology*, vol. 30, ed. Taylor, D.L. & Wang, Y.-L., San Diego: Academic Press (1989), pp. 219-243; Turro, N.J., *Modern Molecular Photochemistry*, Menlo Park: Benjamin/Cummings Publishing Co., Inc. (1978), pp. 296-361 and the Molecular Probes Catalog (1997), OR, USA.

[0054] Preferably, FRET (fluorescence resonance energy transfer) is used as a way of monitoring probes in a sample (cellular or biochemical). The degree of FRET can be determined by any spectral or fluorescence lifetime characteristic of the excited construct, for example, by determining the intensity of the fluorescent signal from the donor, the intensity of fluorescent signal from the acceptor, the ratio of the fluorescence amplitudes near the acceptor's emission maxima to the fluorescence amplitudes near the donor's emission maximum, or the excited state lifetime of the donor. For example, cleavage of the linker increases the intensity of fluorescence from the donor, decreases the intensity of fluorescence from the acceptor, decreases the ratio of fluorescence amplitudes from the acceptor to that from the donor, and increases the excited state lifetime of the donor. Preferably, changes in signal are determined as the ratio of fluorescence at two different emission wavelengths, a process referred to as "ratioing." Differences in the absolute amount of probe (or substrate) cells, excitation intensity, and turbidity or other background absorbencies between addressable wells can affect the fluorescence signal. Therefore, the ratio of the two emission intensities is a more robust and preferred measure of activity than emission intensity alone.

EXAMPLES

EXAMPLE 1 - CONSTRUCTION AND TESTING OF A BALL LENS TRIFURCATED FIBER OPTIC ASSEMBLY

[0055] Arrangements of ball lenses and trifurcated fibers can be tailored to their intended application. To determine the appropriate arrangement of fiber optic bundles and ball lens a series of experiments can be conducted to determine the highest signal to noise ratio, preferred sensitivity, lowest background, preferred field of optical interrogation or excitation or a combination thereof.

[0056] For example, one embodiment of a trifurcated fiber optic assembly adapted for miniaturized sample analysis of a 1 mm well diameter with a variable interrogation layer of approximately 0.1mm to 2.0 mm could comprise the following arrangement. A ball lens made of fused silica material coated with an antireflective coating such as HEBBAR, with a diameter of about 3mm. A trifurcated fiber optic assembly, optically coupled to the ball lens comprising 91 fibers, of which 7 fibers in the center are for excitation, and the remaining fibers are for emission collection. The fiber assembly being about 3mm in diameter and packed into a hexagonal ferrule to maximize packing efficiency and ease of assembly. The emission fibers (42 for each optical property to be measured) are selected so as to maximize collection efficiency, signal intensities and signal to noise or signal to background properties of the assembly. The following table (Table 1) illustrates the effect that the spatial position of the ball lens to the fiber assembly has on signal-to-background ratios. In this example, the interrogation distance of the ball lens to the test fluorescent sample was kept constant as the transmission distance of the ball lens to the fiber optic assembly was varied. Different signal (10 nM fluorescein-10 nM F) to background (BB) ratio's were obtained from which an optimal transmission distance could be selected, which under these conditions was 0.482 mm

Table 1

Emission filter: 535RDF30 (no long pass)				(Signal – background) / background
Sample volume: 2 microliters, hand loaded.				
Ball lenses: Fused silica.				
Distance from lens to plate = 0.			10 nM F Signal	(10 nM F - BB) / BB
Fiber-to-lens (mm)	Empty (mV)	Buffer (BB) Background		
				9
0	10.8	12.4	119	
0.384	9	7.95	117.67	14
0.482	7.8	6.95	130.33	18
0.533	8	7.25	126	16
0.584	7.9	7.15	122	16
0.71	4.9	5.8	34	5

Further subsequent analysis (Table 2) of this particular system demonstrates that the interrogation distance between the test sample relative to the ball lens fiber optic assembly can be relatively widely varied (within the range 0 and 0.152 mm) without significantly impacting signal to background values. Thus, demonstrating one useful aspect of the invention.

Table 2

Distance from fiber to lens = 0.584 mm				
lens-to-plate (mm)	Empty (mV)	Buffer(BB) (mV)	10 nM F (mV)	(10 nM F - BB) / BB
0	7.9	7.15	122	16
0.152	7.8	7.2	126	17
0.203	8.4	8.15	119.5	14
0.305	10	8.9	118	12

In order to analyze the influence of the fiber optic arrangement on Signal to Background measurements, a series of fiber optic bundle assemblies were compared under identical conditions. In this case, a 3mm HEBBAR coated sapphire ball lens was utilized with four different fiber optic assemblies (as shown in FIG. 3). These assemblies contained varying numbers and arrangements of both excitation and emission fibers. Fiber optic bundle assembly performance was assessed by measuring the minimum detectable level (MDL) of a particular dye as described herein.

Table 3

Description of Assembly	MDL in nM of Fluo- rescein	Number of Ex Fibers	Number of Em Fibers	Fiber Assembly Diam- eter
Assembly #1	0.50	7.00	84.00	2.6mm
Assembly #2	0.86	1.00	6.00	1.2mm
Assembly #3	0.50	3.00	16.00	1.5mm
Assembly #4	0.50	7.00	30.00	1.6mm

Surprisingly, as shown in Table 3, assemblies #3 and #4 perform (as shown in FIG. 3) as well as assembly #1. Even

though assembly one is significantly larger than the other assemblies, and contains more fiber optic fibers (FIG 3). This indicates that there is a relationship between fiber optic bundle size and the optimal ball lens size, and that this optimally about equal or 1 to 3 times greater in diameter than the fiber optic assembly. The ball lens can thus aid in reducing the complexity or quantity of fibers required in a fiber optic assembly for optimal detection sensitivity particularly when the need to reduce the size of the fiber optic assembly is important in a miniaturized system.

[0057] In a similar example to above, (Table 4) the fiber assembly is kept constant but the size of the ball lens is varied. In this example, a 3mm diameter coaxial fiber optic — (3mmCoAX) assembly containing 112 fibers arranged with 7 XXF200/210/235T fused silica excitation fibers in the center of the assembly surrounded by 105 XXF200/210/235T fused silica emission fibers is used with three different size sapphire ball lenses, of external diameters of 3mm, 5mm and 10mm. As Table 4 illustrates, sensitivity as measured by MDL determinations, sensitivity improves as ball lens size increases. Surprisingly, sensitivity increases by a factor of 15 in moving from a 3 mm ball lens to a 10 mm ball lens.

Table 4

Different Size Ball Lens	Relative Sensitivity
Experiment Description	Molar equivalents of dye
Glass Bottom plate with Solution Standards	
Cermax 300 w Xenon Lamp, dual excitation and emission filtering	
Sapphire Ball Lens with HEBBAR coating	
PMT- 3mmCoAX -10mmHB-DF	1.125E-12
PMT- 3mmCoAX -5mmHB-DF	1.108E-11
PMT- 3mmCoAX -3mmHB-DF	1.727E-11

Protocols, materials and methods for the experiments herein.

[0058] The minimum detectable level (MDL) was calculated by generating a fluorescein calibration curve that enabled the concentration of fluorescein that was equivalent to 4 times the standard deviation of the buffer blank to be calculated. Buffer blank (BB) measurements are determined from the variance of readings from many buffer blanks and would be affected by well to well variability, positioning artifacts and other errors.

[0059] MDL2 is determined from the variance of repeated measurements of the same buffer blank and presumably would be affected only by the noise of the detector.

[0060] The optical detectors utilized to evaluate fluorescent intensity in the experiments were either a Hamamatsu PMT and associated electronics as described in the Fluorocount instrument or a Hamamatsu HC135-01/100Mhz PMT sensor module with embedded micro controller and RS-232-C interface. This sensor operates in the 360-650 nm range. A Labview™ software interface was written to control the PMT and acquire data. When needed, excitation radiant power was measured using a Newport Corporation 1835-C power meter equipped with a 818-UV NIST traceable silicon photodiode detector. The filters used in these experiments were obtained from ChromaTechnology Corporation or Omega Optical Inc., with the exception of neutral density filters that were obtained from Oriel Corp. In general and except where noted, all experiments were conducted with the Hamamatsu PMT were double filtered on the excitation and emission ends with a 0.2 neutral density filter sandwiched in between the interference filters. The excitation filters were IQ475/40 +0.2 ND+D480/20x. The emission filters were 535DF35t +0.2ND+535DF.

[0061] Three different light supplies were utilized for the experiments and are identified as appropriate in the experimental results section. The first was a Quartz Tungsten Halogen (QTH) light obtained from Cole-Palmer Model # H-41700-00. The second was a Cermax LX-300W xenon Arc with integral parabolic reflector. The third was a 175 watt Xenon Arc lamp with ultra stable power supply from Hamamatsu.

[0062] All of the ball lenses were coated with HEBBAR. Experiments with the Hamamatsu PMT were performed on a Newport Corporation optical bench with Vibration dampening. Certain fixtures and mounts were specially made through local machine shops and others were obtained through Newport Corporation.

[0063] Three types of plates were utilized. The standard plate is a 96 well black top clear bottom polystyrene plate filled with fluorescent standards. The glass bottom plates were specially modified black polystyrene 96 well plates with 175 micron glass bottoms. 384 well black polystyrene glass bottom plates were utilized for the 384 well readings. These

specially modified plates were obtained from polyfiltronics/Whitman.

[0064] The fiber optic assemblies were composed of fused silica coated with a black polyimide coating obtained from Fiberguide. The individual fibers are 200/220/240 in microns in diameter for the core/cladding/coating respectively unless otherwise specified in particular experiments.

Example 2- Sensitivity, and Background Testing of optical assemblies of one embodiment of the invention.

[0065] This example demonstrates the ability of the optical assemblies to achieve uniform illumination of the addressable wells while at the same time avoiding illumination of the sides of the well and the illumination of adjacent wells. This leads to reduced background fluorescent signals caused by reflections from the plate and wells and reduces punch through of excitation light through emission filters into detection system, yet enables high sensitivity detection at two wavelengths.

[0066] This is exemplified by the determinations of minimum detectability of a number of fluorescent standards. For example, the minimum detectable fluorescein level achieved using a detector incorporating the optical system of the invention was better than 50 pM fluorescein in a standard 96 well plate Table 5. Emission was collected at wavelengths centered at both 535 nm and 580 nm. Both a blank solution and a solution containing 2nM fluorescein were measured. The minimum detectable level (MDL) was calculated by generating a fluorescein calibration curve that enabled the concentration of fluorescein that was equivalent to 4 times the standard deviation of the buffer blank to be calculated. Because the detector typically measures changes of brightness within a single well, the standard deviations for readings within the same well at 1 Hz for eight seconds are given. It was found that the plate material also affected the MDL levels. Both buffer and fluorescein statistics were determined from 100 μ L volumes in 40 wells (5 columns of 8 wells) of a 96 well plate. Fluorescein MDL levels measured using 480 ± 10 nm excitation 535 ± 17.5 nm and 580 ± 30 nm emission filters.

Table 5

Plate bottom material	Glass	Glass	Polystyrene	Polystyrene
Emission wavelength	535 nm	580 nm	535 nm	580 nm
MDL (nM fluorescein)	0.0017	0.0085	0.034	0.072

Because the fluorescent dyes typically used with the detector are not excited at fluorescein wavelengths, more relevant standards are the fluorophores 3-glycine chloro-coumarin (3GCC) and rhodamine 101 Table 6. MDL measurements were determined for these fluorescent dyes as described above except that a fluorescent dye solution also containing 25 nM fluorophores 3-glycine chloro-coumarin and 4 μ M rhodamine 101 was used in place of the fluorescein solution

Table 6

Fluorescent dye	3GCC	rhodamine 101
Plate bottom material	Polystyrene	polystyrene
Emission wavelength	460 nm	580 nm
Excitation Wavelength	400 nm	400 nm
MDL (nM fluorescent dye)	0.181	20.8

Two Dye MDL levels measured both excited using a 400 ± 7.5 nm filter. The 3GCC fluorescence was collected using a 460 ± 22.5 nm filter; the rhodamine 101 fluorescence was collected using a 580 ± 30 nm filter. Because 400 nm excitation light is not optimal for the efficient excitation of rhodamine 101, the MDL level for this fluorophore is relatively high when compared to those for 3GCC or fluorescein.

[0067] A desirable feature of the invention is that the fiber optic bundle and ball lens assemblies enable efficient excitation of the addressable wells, as well as the ability to simultaneously measure at least two optical properties. The average measured excitation intensity at 400 nm emerging through each of the fiber optic bundles and ball lens of the invention is 529 ± 75 μ W when using two 400 ± 7.5 nm excitation filters. The light source used was an ILC CXP300 300 watt Xenon arc lamp, with 6.3 mm anti reflection coated fused silica ball lenses at the common ends of each of eight 5.18 mm diameter bundles containing 333 fibers, 111 fibers from each leg of the randomly packed trifurcated bundles.

Light power was measured using a calibrated Newport 1835-C powermeter.

[0068] The use of the trifurcated fibers and ball lens system, and the calculation of an emission ratio significantly reduces experimental noise, eliminates relative excitation variability between the 8 fiber optic assemblies in the detector and leads to smaller C.V.s and improves the dynamic range of FRET based assays. A major additional advantage is the removal of addition artifacts to enable continuous measurements during reagent addition. In these phenomena, intensities of cells loaded with fluorescent dye often decline upon reagent addition. This decline in intensity may be due to some cells being washed from the detection area during addition and mixing of reagents. By taking the emission ratio at two separate wavelengths these artifacts are eliminated. In the data set below Table 7, a mammalian neuronal cell line was loaded using a FRET based fluorescent dye system. In this example, the majority of the emission change was in the 460 nm channel. For this experiment, monolayers (e.g. about 5 to 50 micrometers) of mammalian cells were plated into the first 6 columns of a 96 well plate. The emission intensities measurements were made at two wavelengths and the ratio determined for 35 seconds at 1Hz for each of the 8 wells in a column. Reagent solutions were added following the 12th read of each column. In this example, test cells stimulated by depolarization by addition of 100uL high potassium solution (90mM K). Control cells received normal Hank's buffered saline solution (HBS) without high potassium to test for addition artifacts. Both intensity data and emission data were normalized versus basal levels to account for well to well variations in cell number or loading brightness and normalized basal levels prior to reagent addition. This enables direct comparisons between intensity data and ratiometric data.

Table 7

Comparison of ratio versus non-ratio measurements (Data Normalized to Initial Values)			
Non-ratio measurements		Ratio measurements	
460 alone		Emission Ratio (460/580)	
HBS		HBS	
AV	91.7%	AV	99.2%
SD	4.0%	SD	1.4%
CV	4.4%	CV	1.4%
HiK		HiK	
AV	139.3%	AV	155.6%
SD	6.3%	SD	4.9%
CV	4.5%	CV	3.1%
Difference	47.6%	Difference	56.5%

[0069] As can be seen in Table 7 both the standard deviations (SD) and coefficient of variation (CV) are about 30 % lower for the ratiometric data (1.4% compared to 4.4% for HBS controls). There is also an addition artifact (91.7% of basal) in the intensity data but not in the emission data (99.2% of basal) for the control HBS additions. Because the emission ratio data factors both the increase in intensity at 460nm and the slight decrease in intensity at 580nm upon depolarization with HiK solution, the dynamic range of the emission ratio data is larger than that of the single intensity data. Statistics were determined from 24 wells (3 rows of 8 wells).

EXAMPLE 3- Determination of Na⁺ Dependent Depolarization in mammalian Cells

[0070] An advantage of the use of the optical assemblies of the invention is the ability to rapidly measure two wavelengths simultaneously thereby enabling the rapid analysis of cellular responses. In the field of voltage sensing, the use of rapid depolarization measurements has several significant advantages over earlier relatively slow depolarization approaches that are subject to artifacts and reduce throughput of the assay. The use of the device thus allows the development of sensitive and rapid assay systems for membrane voltage measurements in whole cells. As shown in Table 8, these assays are highly sensitive, reliable and able to discriminate relatively small changes in membrane potential with high precision.

[0071] Mammalian neuronal cells were grown in F12 complete medium supplemented with 20 % fetal bovine serum. Prior to experiments cells were washed twice with sodium free buffer (140mM N-methyl-D-glucamine, 10 mM HEPES,

pH 7.2, 0.34 mM Na₂HPO₄, 0.4 mM MgCl₂, 0.5 mM KH₂PO₄, 5.37 mM KCl, 1.26 mM CaCl₂, 2g/L D-glucose). The cells were then harvested using calcium and magnesium free buffer and washed once. The cells were then loaded with the fluorescent dye CCI-DMPE (4 μ M for 30 minutes at room temperature) and washed in sodium free buffer. The fluorescent dye DiSBAC₂ was then added to the cells, after 30 minutes the plates were loaded onto the device of the invention. All wells treated with a channel opener to open Na⁺ channels and maintained in low Na⁺ solution. Each well contained approximately 10⁵ cells. The average, standard deviation, and standard error of the mean are given in the for cells treated with three different experimental conditions, low extracellular sodium (0 Na⁺, buffer with high sodium (HBS) and buffer with high sodium in the presence of a sodium channel blocker (HBS-TTX). The results (Table 8) show that the change in membrane voltage exerted by the change in extracellular sodium ion concentration can be accurately measured using a device comprising the present invention.

Table 8

	0 Na ⁺	HBS	HBS-TTX
AV	99.5%	130.2%	98.9%
SD	0.9%	4.3%	0.9%
C.V.	0.9%	3.3%	0.9%
Difference	N/A	30.7 %	-0.6%

Example 4. Determination of Dose Response Relationships

[0072] The large ratio changes observed with this method enable the creation of highly reproducible assays and provide signals large enough for dose response curves to be generated. Furthermore because the device can acquire data continuously, the responses from the individual wells can be viewed as a function of time. FIG 8A shows the real time changes in voltage for individual wells.

[0073] The cells were stained and handled as described in Table 8. All wells contained a sodium channel agonist. Traces show the effect of different doses of an anesthetic RS-105914-197 on blocking Na⁺ channel activity in the neuronal cells. FIG 8B shows the dose response of the anesthetic RS-105914-197 for blocking sodium channel activity using the device of the invention. The data represents the average of 4 wells and the error bars represent the CV value. 1 mM of the drug completely blocks the Na⁺ induced depolarization. These results with error analysis are summarized in Table 9. The results show that a device of the invention provides a sensitive, accurate and reproducible method of measuring relatively small changes in fluorescence measurements.

Table 9

	Mean	S.D.	C.V.
0 mM RS-105914-197	186.4%	3.7%	2.0%
0.1 mM RS-105914-197	172.2%	8.2%	4.7%
0.3 mM RS-105914-197	117.7%	5.6%	4.8%
1.0 mM RS-105914-197	100.5%	2.6%	2.6%

Example 5 - Screening for Antagonists

[0074] To test whether it would be possible to identify antagonists on a single plate assay in a screening format, a protocol was set up. This protocol was designed such that compound additions were made from a chemical multiwell plate to the test plate, and the wells read continuously during compound addition FIG.9 demonstrates the use of the device to identify antagonists in a screening mode. The results show ratio vs well number for the assay run in antagonist screening mode. End ratio values were averaged as in FIG. 9. A test antagonist (100 μ M) was used to test screening sensitivity. Vehicle control wells had an equivalent final concentration of DMSO as the test antagonist treated wells. Negative controls received an addition of buffer instead of agonist. In this experiment, cells (HEK-293) were washed with assay buffer (160mM NaCl, 10 mM HEPES, pH 7.4, 0.34 mM Na₂HPO₄, 0.4 mM MgCl₂, 0.5 mM KH₂PO₄, 5.37 mM KCl, 1.26 mM CaCl₂, 2g/L D-glucose) and loaded with the fluorescent dyes CC2-DMPF and DiSBAC₂ as described

in Table 8.

Publications

[0075] All publications, including patent documents and scientific articles, referred to in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication were individually incorporated by reference.

[0076] All headings are for the convenience of the reader and should not be used to limit the meaning of the text that follows the heading, unless so specified.

Claims

1. A method of simultaneously measuring at least two optical properties of emitted light from at least one sample in a plurality of addressable wells of a multiwell plate comprising the steps of,

- iv) aligning a plurality of addressable wells of a multiwell plate to a plurality of ball lenses;
- v) directing electromagnetic radiation substantially coaxially through the symmetry axis of each of said plurality of ball lenses,
- vi) detecting the emitted light focused by said plurality of ball lenses from said at least one sample.

2. The method of claim 1, wherein said electromagnetic radiation is directed to said plurality of ball lenses by at least one laser.

3. The method of claim 1, wherein said electromagnetic radiation is directed to said plurality of ball lenses by at least one fiber optic bundle.

4. The method of claim 1, wherein said emitted light focused by said plurality of ball lenses is directed to at least one detector through at least one fiber optic bundle.

5. A device, comprising:

- i) a liquid handler comprising at least one pipetting tip, said at least one pipetting tip comprising programmable control of aspiration from a first plurality of addressable wells of a first multiwell plate and programmable control of dispensation into a second plurality of addressable wells of a second multiwell plate,
- ii) an optical detector module comprising at least one detector, said at least one detector being in optical connection to said second plurality of addressable wells of said second multiwell plate and, said detector module simultaneously detecting at least two optical properties from each well of said second plurality of addressable wells of said second multiwell plate,
- iii) a programmable moving conveying surface to align said first multiwell plate and said second multiwell plate to said liquid handler, and said detector module, and to move said first multiwell plate and said second multiwell plate into and out of said device,
- iv) a data processing and control module for coordinating the operation of said automatic measuring device, wherein said data processing and control module coordinates said programmable moving conveying surface to move said second plurality of addressable wells of said second multiwell plate to said liquid handler, wherein said liquid handler simultaneously dispenses into said second addressable wells of said second multiwell plate and said detector module simultaneously measures at least two optical properties from each well of said second plurality of addressable wells of said second multiwell plate, and wherein, said data processing and control module collects data from said detector module.

6. The device of claim 5, wherein said data processing and control module intermittently collects data from said detector module.

7. The device of claim 5, wherein said liquid handler dispenses into said second addressable wells of said second multiwell plate and there is a predefined delay before said detector module simultaneously detects at least two optical properties from each well of a plurality of said second plurality of addressable wells of said second multiwell plate.

8. The device of claim 5, wherein the optical property is light emission at a particular wavelength.

9. The device of claim 5, further comprising a optical irradiation module comprising at least one light source, wherein said at least one light source irradiates said second plurality of addressable wells of said multiwell plate.
- 5 10. The device of claim 5, wherein said at least one light source irradiates said second plurality of addressable wells of said multiwell plate intermittently.
11. The device of claim 5, wherein, said at least one light source is programably controlled to irradiate said second plurality of addressable wells of said multiwell plate at predefined times.
- 10 12. The device of claim 5, wherein said detector module further comprises at least one fiber optic bundle.
13. The device of claim 8, wherein said at least one fiber optic bundle comprises at least one trifurcated fiber optic bundle.
- 15 14. The device of claim 5, wherein said detector module further comprises at least one ball lens.
15. The device of claim 14, wherein said at least one ball lens is formed of a material selected from the group consisting of glass, fused silica, quartz and sapphire.
- 20 16. The device of claim 15, wherein said at least one ball lens further comprises an anti-reflective coating.
17. The device of claim 14, wherein said detector module further comprises at least one trifurcated fiber optic bundle, said at least one trifurcated fiber optic bundle having a diameter that is one third the diameter of said at least one ball lens.
- 25 18. The device of claim 14, wherein said detector module further comprises at least one trifurcated fiber optic bundle, said at least one trifurcated fiber optic bundle comprising at least one central optical fiber that is in direct optical communication with said at least one light source, and wherein said at least one central fiber optic bundle is coaxially aligned to said at least one ball lens.
- 30 19. A device, comprising:
 - i) an optical detector module comprising at least one detector, said at least one detector being in optical connection to a plurality of addressable wells of a multiwell plate and, said detector module simultaneously detecting at any instant, at least two optical properties from each well of said plurality of addressable wells of said multiwell plate.
 - 35 ii) a optical irradiation module comprising at least one light source, wherein said at least one light source irradiates said plurality of addressable wells of said multiwell plate,
 - iii) a programmable moving conveying surface to align said plurality of addressable wells of said multiwell plate to said detector module, and to move said plurality of addressable wells of said multiwell plate into and out of said device,
 - 40 iv) an integration and control module for coordinating the operation of said automatic measuring device, wherein said data processing and control module coordinates said programmable moving conveying surface to move said plurality of addressable wells of said multiwell plate to said detector module,
 - 45 wherein said detector module simultaneously detects at least two optical properties from each well of said second plurality of addressable wells of said second multiwell place and, wherein said data processing and control module collects data from said detector module.
20. The device of claim 19, wherein said data processing and control module intermittently collects data from said detector module.
- 50 21. The device of claim 19, wherein the optical property is light emission at a particular wavelength.
22. The device of claim 19, wherein said at least one light source irradiates said plurality of addressable wells of said multiwell plate intermittently.
- 55 23. The device of claim 19, wherein, said at least one light source is programably controlled to irradiate said plurality of addressable wells of said multiwell plate at predefined times.

24. The device of claim 19, wherein said detector module further comprises at least one fiber optic bundle.
25. The device of claim 24, wherein said at least fiber optic bundle comprises a trifurcated fiber optic bundle.
- 5 26. The device of claim 19, wherein said detector module further comprises at least one ball lens.
27. The device of claim 26, wherein said at least one ball lens is formed of a material selected from the group consisting of glass, fused silica, quartz and sapphire.
- 10 28. The device of claim 27, wherein said at least one ball lens further comprises an anti-reflective coating.
29. The device of claim 26, wherein said detector module further comprises at least one trifurcated fiber optic bundle, said at least one trifurcated fiber optic bundle having a diameter that is one third the diameter of said at least one ball lens.
- 15 30. The device of claim 26, wherein said detector module further comprises at least one trifurcated fiber optic bundle, said at least one trifurcated fiber optic bundle comprising a central optical fiber bundle that is in direct optical communication with said at least one light source, and wherein said central fiber optic bundle is coaxially aligned to said at least one ball lens.
- 20 31. The device of claim 26 wherein said at least one ball lens is about three times larger in diameter than said addressable wells of said multiwell plate.
32. The device of claim 26 wherein said at least one ball lens is about the same diameter as said addressable wells of said multiwell plate.
- 25 33. An optical assembly, comprising a ball lens and a trifurcated fiber adapted for dual optical interrogation and in optical communication with said ball lens.
- 30 34. The optical assembly of claim 33, wherein said trifurcated fiber comprises a first optically isolated emission bundle to collect light, second optically isolated emission bundle to collect light, and an excitation bundle.
- 35 35. The optical assembly of claim 34, wherein said ball lens is separated from said trifurcated fiber by a transmission space.
36. The optical assembly of claim 35, wherein said ball lens comprises a sapphire material.
37. The optical assembly of claim 36, wherein said ball lens comprises an anti-reflective coating.
- 40 38. The optical assembly of claim 33, wherein said trifurcated fiber comprises a first plurality of emission bundles for receiving light of a first wavelength and second plurality of emission bundles for receiving light of a second wavelength and said first plurality of emission bundles and said second plurality of emission bundles are randomly distributed in plurality of excitation bundles.
- 45 39. The optical assembly of claim 33, wherein said trifurcated fiber comprises a first set of bundles for transmitting light of a first wavelength and second set of bundles for transmitting light of a second wavelength and third set of bundles for transmitting light of a third wavelength.
- 50 40. The optical assembly of claim 39, wherein said trifurcated fiber is separated from said ball lens by a transmission space of about .1mm to 1mm.
41. The optical assembly of claim 35, wherein said ball lens comprises either sapphire material or a silica material.
42. The optical assembly of claim 39, wherein said first set of bundles and said second set of bundles are coaxially arranged with respect to said third set of bundles.
- 55 43. An optical detection system, comprising:

- a) a light source that launches at least one predetermined wavelength of light,
- b) sample holder,
- c) a ball lens at a predetermined interrogation distance from said sample holder,
- d) a trifurcated fiber adapted for dual optical interrogation and in optical communication with said ball lens, and
- e) a detector that detects light of at least one desired wavelength and in optical communication with said ball lens.

44. The optical detection system of claim 43, wherein said trifurcated fiber comprises a first plurality of emission bundles for receiving light of a first wavelength and second plurality of emission bundles for receiving light of a second wavelength and said first plurality of emission bundles and said light source launches at least one predetermined wavelength of excitation light at said sample holder.
45. The optical detection system of claim 43, wherein said ball lens is at a predetermined transmission distance from said trifurcated fiber and further comprising at least one positioner to controllably change said predetermined transmission distance.
46. The optical detection system of claim 43, further comprising at least one positioner to controllably change said predetermined interrogation distance.
47. The optical detection system of claim 43, further comprising a liquid handling unit to dispense liquids into addressable wells.
48. The optical detection system of claim 43, further comprising a light activation system for triggering liquid handling unit to dispense liquids into addressable wells.
49. The optical detection system of claim 43, wherein said light source launches light through said trifurcated fiber to the location at least one addressable well in a sample in said sample holder to monitor epifluorescence.
50. The optical detection system of claim 49, further comprising a computer control system to manage interrogation of a sample.
51. The optical detection system of claim 49, further comprising a sample transfer system to transfer at least one sample platform to said sample holder.
52. The optical detection system of claim 49, wherein said sample holder further comprises a positioning system.
53. The optical detection system of claim 43, wherein said ball lens is at a predetermined transmission distance from said trifurcated fiber that approximately corresponds to a focal length.
54. The optical detection system of claim 43, wherein said trifurcated fiber comprises an end and said end is generally at a focal plane of said ball lens.
55. The optical detection system of claim 43, wherein an object to be interrogated is generally at a focal plane of said ball lens.
56. An optical fiber assembly, comprising a trifurcated fiber comprising a first plurality of emission bundles for receiving light of a first wavelength and second plurality of emission bundles for receiving light of a second wavelength and said first plurality of emission bundles and said second plurality of emission bundles are non-randomly distributed in plurality of excitation bundles.
57. The optical fiber assembly of claim 56, wherein said first set of bundles and said second set of bundles are coaxially arranged with respect to said third set of bundles.
58. The optical fiber assembly of claim 56, wherein said first set of bundles is coaxially arranged with respect to said second set of bundles.
59. A method of identifying a useful chemical, comprising interrogating a sample comprising a chemical of interest with one of the above claimed devices, and detecting the activity of said chemical from optical signals from said device.

60. A method of development for a therapeutic, comprising interrogating a sample comprising a chemical with of interest one of the above claimed devices, detecting the activity of said chemical from optical signals from said device, administering said chemical or a chemical derived from the structure or activity of said chemical to a cell, invertebrate or mammal, assessing a therapeutic effect of said administering and optionally adding a suitable pharmaceutical carrier to said chemical for administration into a vertebrate or human.

61. A chemical identified by a process of detecting optical signals from one of the above claimed devices, wherein said chemical is in a sample interrogated by said device.

62. A pharmaceutical composition, comprising a pharmaceutical acceptable carrier and a chemical identified by detecting optical signals from one of the above claimed devices, wherein said chemical is in a sample interrogated by said device.

FIG 1

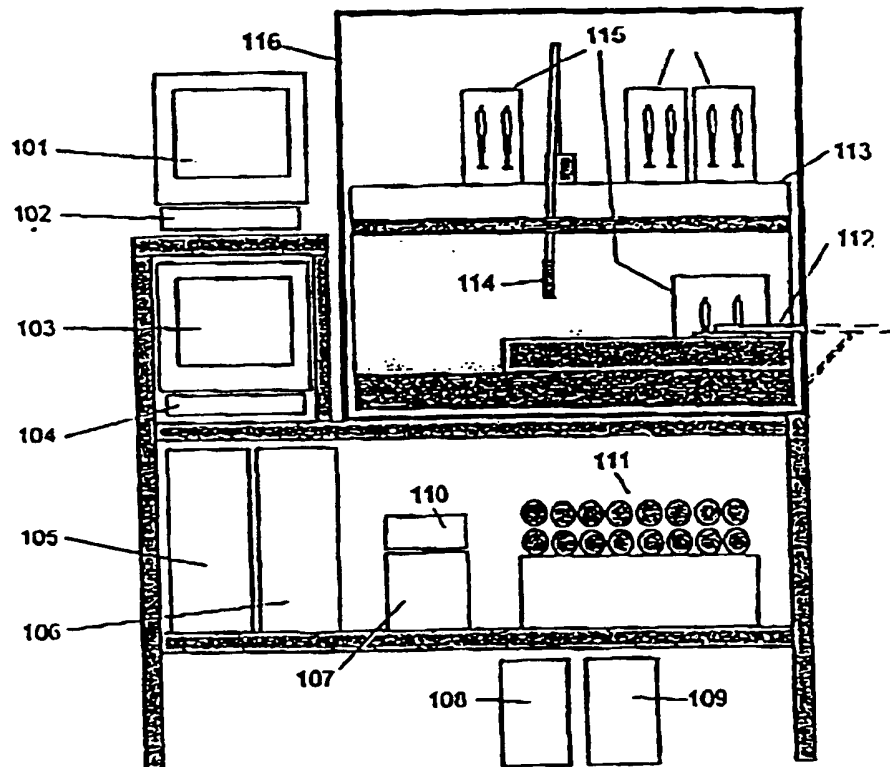


FIG 2

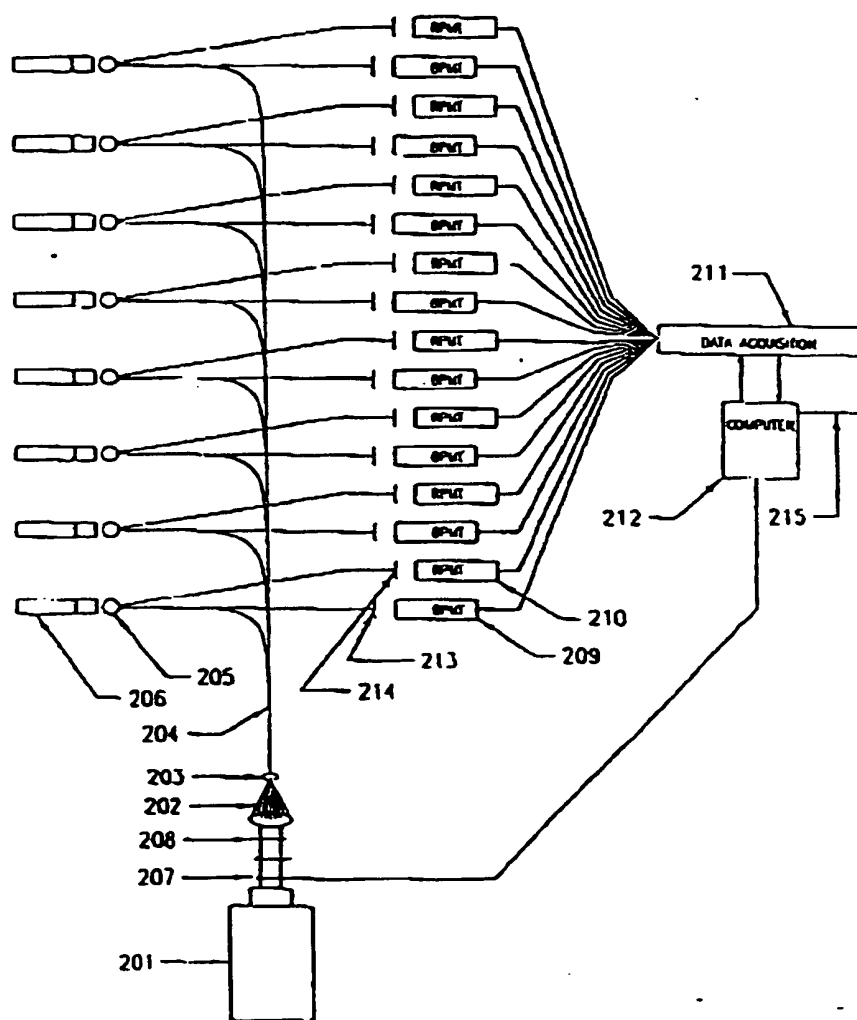
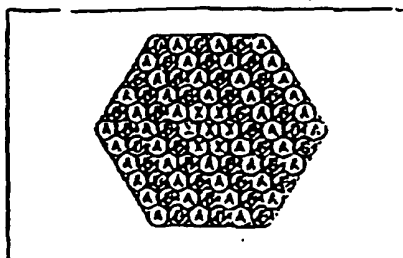
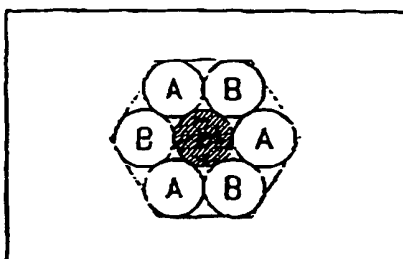


FIG 3

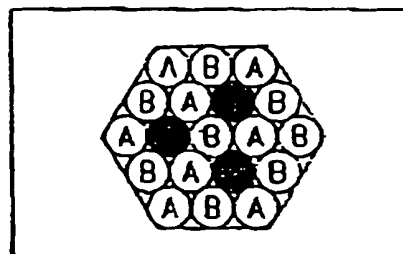
Assembly 1



Assembly 2



Assembly 3



Assembly 4

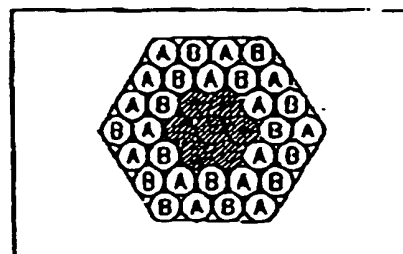


FIG 4

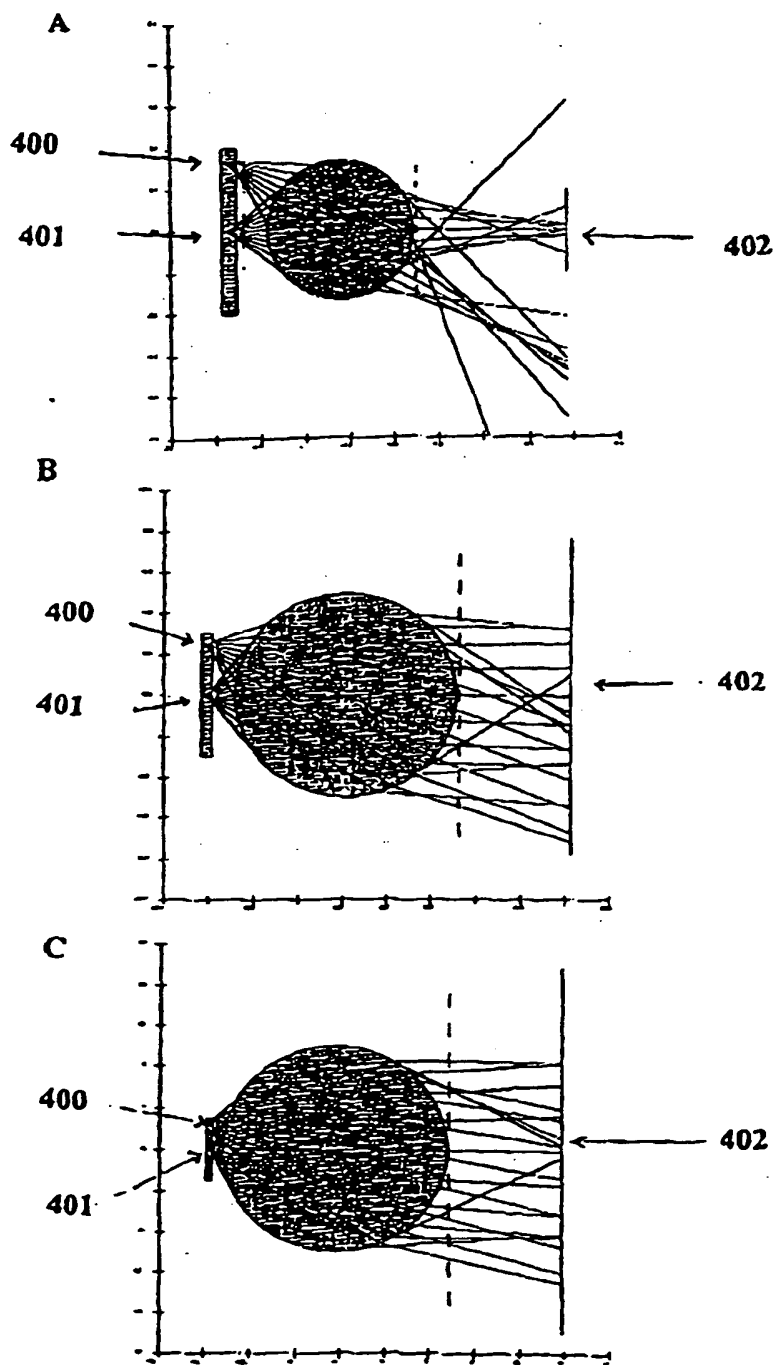


FIG 5

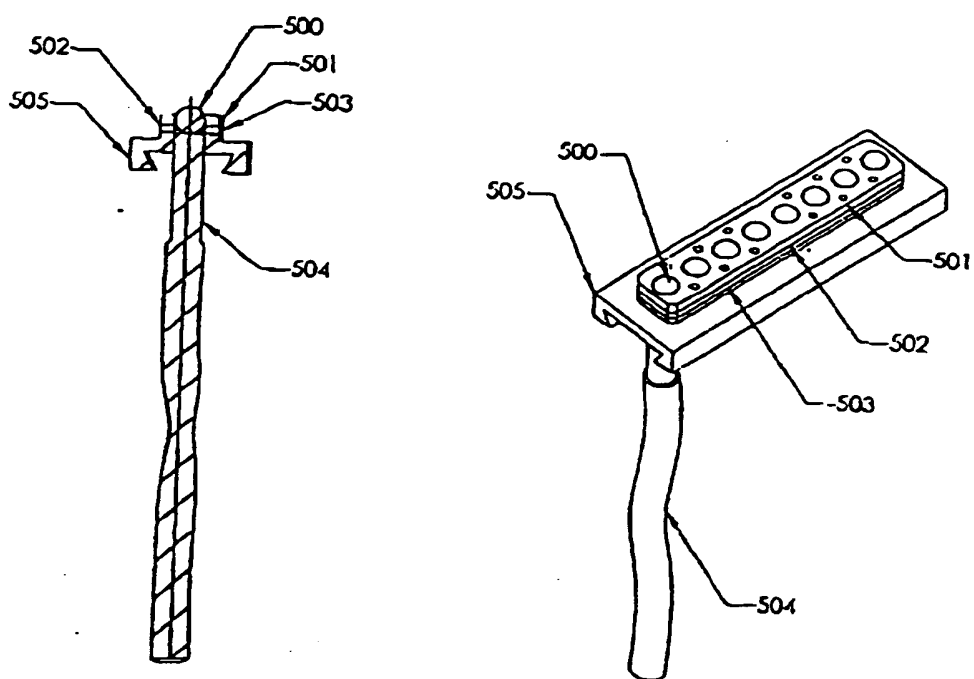


FIG 6

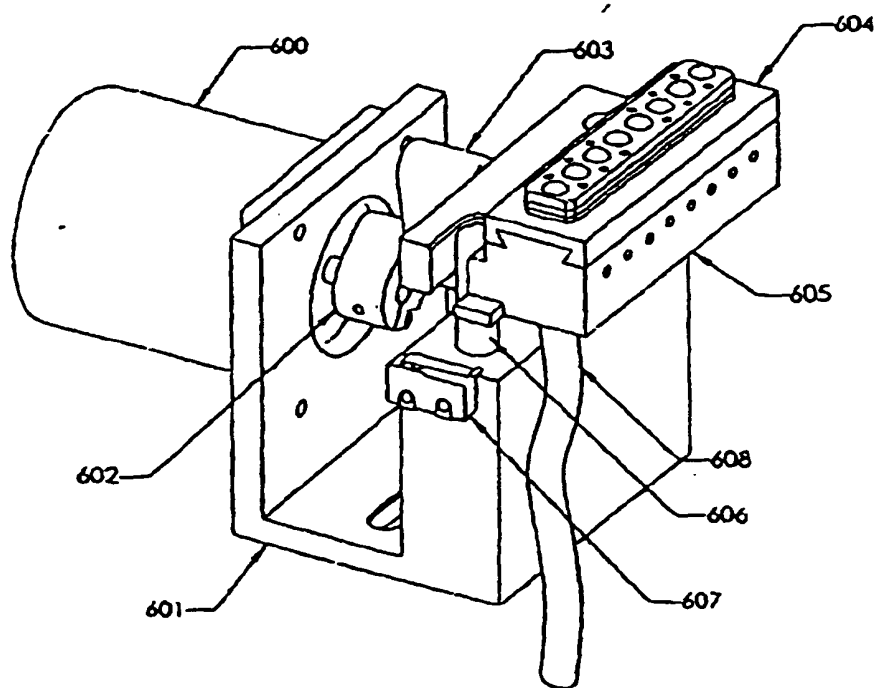


FIG 7

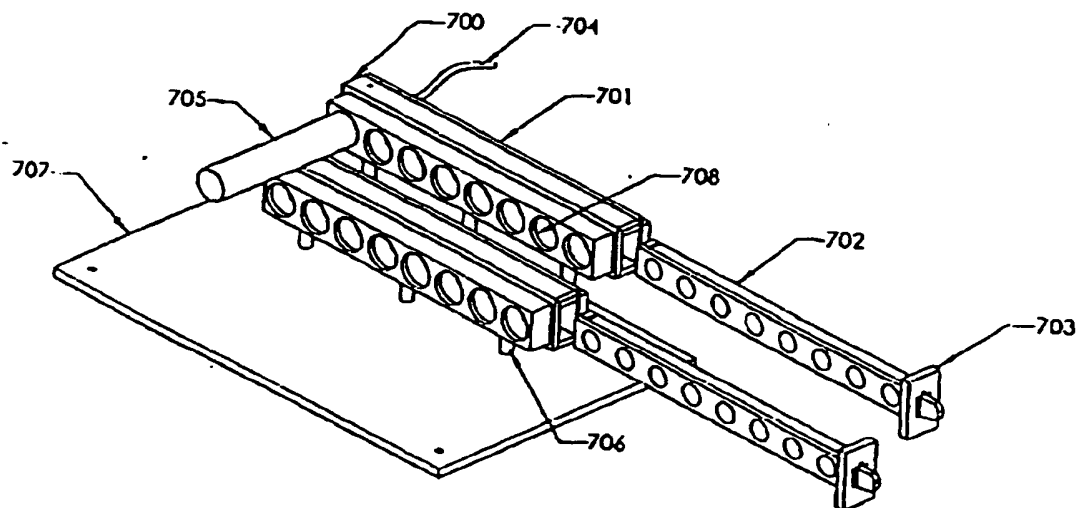


FIG 8A

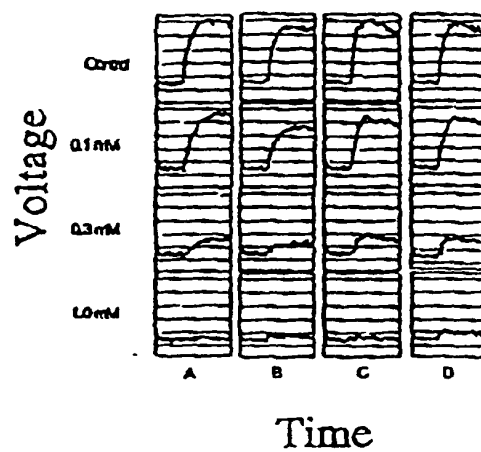


FIG 8B

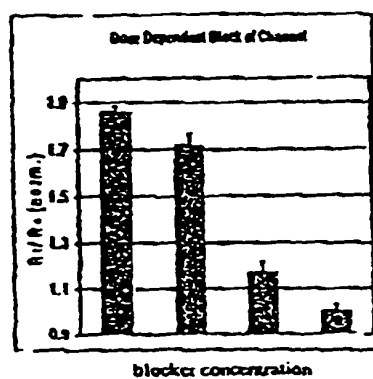
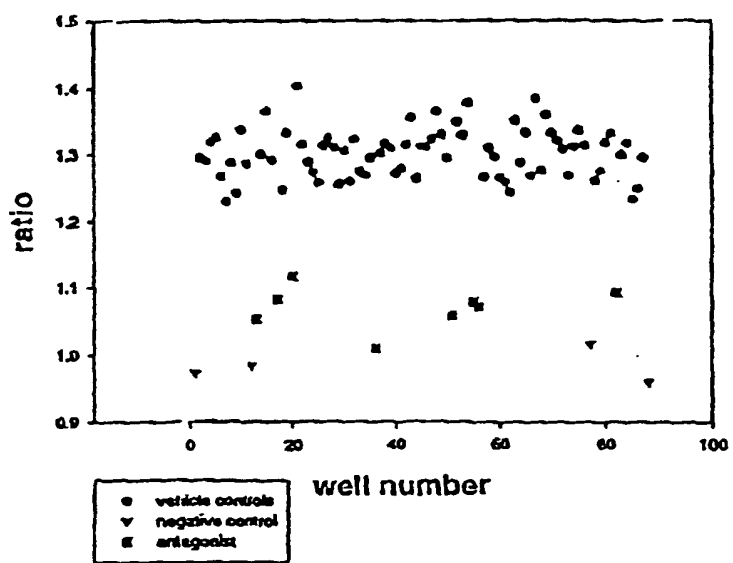


FIG 9



(19)



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(11)

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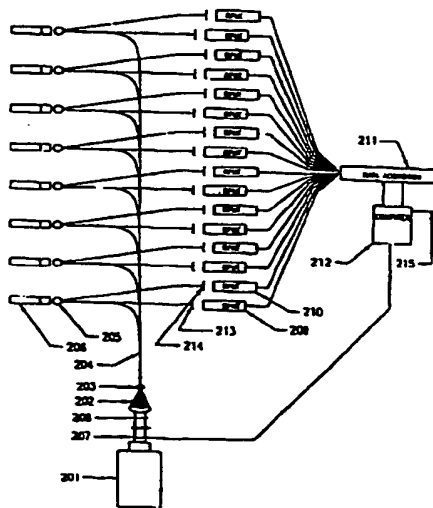
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(54) **Detector and screening device for ion channels**

(57) The invention provides for a detector assembly, fiber assembly and screening system for optical measurements.

FIG 2



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Application Number
EP 99 11 3933

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The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 14 January 2000	Examiner KRAMETZ E.M.
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>			

EPO FORM 1503 03/02 (P04C01)



European Patent
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EUROPEAN SEARCH REPORT

Application Number
EP 99 11 3933

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
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A	--- EP 0 230 679 A (DOW CHEMICAL CO) 5 August 1987 (1987-08-05) * column 3, line 35 - line 45 * * figure 1 *	56-58	
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The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (Int.Cl.7)
Place of search THE HAGUE		Date of completion of the search 14 January 2000	Examiner KRAMETZ E.M.
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>			

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Application Number

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CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

- ☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):
- ☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

- ☒ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- ☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.
- ☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:
- ☐ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:



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LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 99 11 3933

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. Claims: 1-55, 59-62

Method and device for simultaneously measuring at least two optical properties of emitted light from a multiwell plate using ball lenses.

2. Claims: 56-58

Optical fiber assembly comprising a trifurcated fiber.

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 99 11 3933

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
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14-01-2000

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